FINAL No. 1 *REPORT ON*

CONTRACT NO DA -92-557-FEC-57507

INCLUSIVE DATES 15 April, 1964 *TO* 14 April, 1965

SUBJECT OF INVESTIGATION

STUDIES ON EXPERIMENTAL SHIGELLOSIS
SPECIAL REFERENCE TO
SPECIAL CHARACTERISTICS OF
SHIGELLA SPECIES AND THEIR ACTION
UPON LIGATED LOOP

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Office of the Chief of Research and Development
United States Army
APO 343

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CONTRACT No. DA-92-557-FEC-37307
INCLUSIVE DATES: 15 April 1964 to 14 April 1965.

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ABSTRACT

The serological, biochemical and enzymic specificities of shigella associated with virulence were sought after in conjunction with the ability to induce changes in the ligated intestinal loop of rabbits. The lactose or sucrose positive shigella were isolated from patients and the correlation of the ability to ferment lactose and sucrose and the ability to induce changes in the loop were examined. The ability to ferment lactose seems to associated with virulence but antigens seems more closely associated with virulence. The ability to agglutinated by acid which was said to be associated with virulence was not associated with the ability to induce changes. The pathological changes did not associated with the number of shigella recovered after 24 hours of inoculation, if the number was exceeded 10^8. Shigella was mutated to non-virulent mutants several hours after inoculation and some of shigella recombined with residential organisms and became lactose positive or shigella without shigella antigens. These organisms had weaker ability to induce changes. But on the other hand, there were residential organisms which enhanced the pathological changes in the loop.

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1. PURPOSE OF THE RESEARCH.

Dysentery is still a very important disease throughout the world. This is especially true in the underdeveloping countries and in places where a group of people live closely together. The use of antibiotics in the treatment of this disease has certainly lowered the death rate, but with the developmeny of resistant mutants, the treatment and control of this disease are becoming more complicated. Therefore, an effective preventive measure is necessary for the control of this disease.

It was unfortunate that the investigations in this field were handicapped by the unsuccessful animal experiments. A few years ago, Formal et al succeeded in infecting guinea pigs by oral administration of Shigella species following pretreatment of the animals with carbon tetrachloride. However, animals infected by this method still died from septicaemia, and, by this method alone we cannot expect eo solve the problem of dysentery. More recently Formal et al and Imaizumi et al succeeded in infecting monkeys by oral administration of shigella. Monkeys thus infected show the typical dysentery smptoms and the pathological changes. But monkeys are too expensive to use for analytical study of dysentery infection.

By the use of a modified technique of the ligated intestinal loop method, which was first developed and tried by De on Vibrio cholera, we secceeded in producing pathological changes of the loop by introduction of Shigella species. Histologically these changes are in analogy to that of human dysentery. Therefore, we think this method is best suited for use in the analytical study.

The purpose of the research is to determine the serological, biological and enzymatic specificities of Shigella species as well as the factors influencing the infection, such as mutation of organisms, in conjunction with the pathological changes induced in the loop.

2. THE METHOD OF PREPAIRING THE LIGATED INTESTINAL LOOP.

Rabbits are starved for 24 hours before operation. The animals are then aesthetized with Sodium thiopental and an incision is made at the center of the abdomen. The small intestine is brought out to the operational field and ligated at two places; the length will be determined by the number of loops required for the experiment. The rumen of the loop is washed with sterile saline, and holes, which are opened by the insertion of a needle and a glass tube for wasting, are closed by ligation. Then the portion of the loop near the ligated holes are separated by ligation. Two to four loops are made from this washed loop, each having a length of about 12 cm, and the organisms to be examined are injected with the aid of a syringe into each loop. The portion of the loop where injection is made is separated

by ligation. Loops will be replaced in their original site and abdomen closed.

This modified technique is different from the original De's technique in two points. The first is that the inside of the loop is washed with saline, so that the contents of the loop including normal flora are washed out and the organisms introduced will have better chance of proliferation and infection. The second is that the portions of the loop which received injuries either by incision or by insertion of needle are separated by another ligation, so that the non-specific inflamations caused by the injuries of the loop are eliminated.

Rabbits are sacrificed after 24 hours of operation and any macroscopic changes occuring at the loops are recorded. The fluid which accumulated in the loop is drawn out aseptically with syringe and examined for its character and also plated on agar plates for viable count. The loops are then excised for histological examinations. The degree of the changes of the loop is recorded as follows:

+++= Macroscopically the loop is swollen to its maximum, with or without reddening by capillary expansion. The character of the fluid inside the loop is mostly pus with or without blood. Histologicalyy epithel cells are destroyed and it is almost impossible to differentiate mucosa from submucosa because of a strong bleeding. Epithel cells, blood and cells from infiltration are becoming the exudate of the rumen.

++= The loop is swollen almost to its maximum with or without capillary expansion. Fluid of the loop consists of pus and serous fluid without blood. The hight of lamina propria is very low, epithel is destroyed in some part and the order of the cells is irregular. Mucosa and submucosa are swollen by oedema and cell infiltration is prominent. Partial blooding present.

+= The loop is swollen without capillary expansion. Fluid of the loop contains a little pus. Epithel cells are a little out of order. In lamina propria and in submucosa infiltration of the cells are seen.

-= No macroscopic and microscopic change is seen.

3. EXAMINATION OF SPECIFIC CHARACTER OF SHIGELLA AND ITS CORRELATION TO THE ABILITY TO INDUCE CHANGES IN THE LOOP.

It is well known that the organisms isolated from patients are rich in type-specific antigens, and it is also said that the newly isolated organisms have a strong agglutinability to acid at around pH 2.95. Organisms which have such characteristics are said to have strong virulence to mice, which imply that these characters are the

necessary factors for infection and the cause of dysentery in man. Therefore, we examined the ability of these characters to induce changes in the loop and also sought for other factors of the organisms in conjunction with the ability to induce changes in the loop.

a. Isolation of organisms.

Stool specimen of the patients diagnosed as dysentery were collected from the Toshima and Ebara Municipal Hospital in Tokyo and streaked on SS agar and BTB lactose plates. Plates were incubated for 16-24 hours at 37C, then ten (10) each of lactose negative and positive colonies were isolated. Strains thus isolated were numbered and stored in semi-solid media and used throughout the experiments.

b. Biochemical and serological examinations.

(1) Biochemical reactions.

Fermentation of lactose, sucrose, arabinose, rhamnose, raffinose, mannitol, dulcitol, adonitol, inositol, sorbitol, and salicin was examined. Most of strains showed typical fermentation reactions but some of them showed atypical reactions. Eight (8) out of 222 strains examined fermented lactose after 3 to 7 days incubation, and twenty eight (28) strains fermented sucrose. These strains do not belong to shigella according to the definition, but, because these strains were isolated from typical dysentery cases and except lactose and sucrose fermentation those strains showed the same biochemical and serological reactions as shigella, and also because it is possible to induce lactose and sucrose fermentation ability to shigella from E. coli by genetic recombination, we included those strains for further studies as a mutant of shigella.

(2) Serological reactions.

Using type-specific and group sera, we typed the 222 strains. The sero-type of strains is as follows:

Sero-type	Number of Cultures	Sero-type	Number of Cultures
Sh. flexneri 1a	3	Sh. flexneri 4	7
1b	10	var. X	2
2a	56	var. Y	5
2b	25	Sh. sonnei I	55
3a	59		

All of the strains showed typical serological reactions and the distribution of the sero-types was almost the same as that of reported by others in Japan.

(3) Acid-agglutination reaction.

Acid agglutination reaction was carried out using Michaelis' lactic acid buffer solution. Preparation method and pH used for the experiments are shown below.

(a) Preparation method and pH.

	Tube No.					
	1	2	3	4	5	6
1 N. NaOH (ml)	5	5	5	5	5	5
1 N. Lactic acid (ml)	7.5	10	15	25	45	85
Distilled water (ml)	87.5	85	80	70	50	10
pH	4.15	3.85	3.55	3.25	2.95	2.65

(b) Method of acid-agglutination.

A strain to be tested was cultured on nutrient agar plate and incubated at 37C for 18 hours. Organisms were harvested and suspended in distilled water, so as to the suspension contains about 6×10^9 organisms per ml. One ml each of the suspension was added to each tubes containing two (2) ml of buffer solution of various pH. Tubes were incubated at 37C for 2 hours, then examined whether organisms were agglutinated or not. Results were recorded as follows:

+++= All of the organisms were agglutinated and supernatant clear.
++= 50-90% of the organisms were agglutinated.
+= Weak agglutination.
±= Very weak agglutination.
-= No agglutination.

(c) Result.

Eight (8) out of 91 Sh. flexneri strains tested showed +++ reaction in one or more tubes. Twenty (20) strains showed ++ reactions, 18 strains showed + reaction, and 45 strains showed - reaction. Ratio of acid-agglutination positive strains among newly isolated strains was 50.5%, and this does not agree with the report of Anzai et al who has reported that more than 80% of the newly isolated strains were agglutinated by acid. Moreover, in our experiment agglutination

occured at several pH and there was no difference in its degree of agglutination between tubes. This also does not agree with Anzai's which stated that the agglutination occured strongly at pH 2.95. One of the conditions which differ from Anzai's experiment was the resistance of the organisms against antibiotics. Fifty (50) per cent of the organisms now isolated from patients are resistant to antibiotics of some kind while the resistant organisms occupied only a minor part several years ago. Therefore, we investigated the correlation of antibiotic resistance and acid-agglutination ability, but we could not find any correlation.

c. Correlation of biochemical and serological characteristics to induce changes in the loop.

(1) Correlation of biochemical characteristics and the pathological changes.

We examined the ability of lactose positive shigella to induce changes in the loop. Of 8 lactose positive strains, 1 strain showed positive pathological changes, while remaining 7 strains showed no change. (Table 1)

Formal et al reported that when lac locus was introduced in shigella from E. coli, the ability to cause dysentery in animals was lost. In our recombination experiments too, the ability to induce changes in the loop was lost when lac locus was introduced into shigella, but in those cases the type-specific antigens were also lost and we could not decide whether the loss of pathogenicity was associated with the introduction of lac locus or change in type-specific antigens.

The lactose positive strains we isolated were antigenically specific to their types, so it is most probable that the locus controlling the pathogenicity is closely linked to lac locus, although we were still testing either the lac positive property is episomal origin or derived from mutation at lac loci.

Of 28 sucrose positive strains, 14 strains (50%) showed positive pahtological changes. Therefore, mutation in this fermentation does not seem to affect the ability to induce changes in the loop.

No correlation was found between any of other biochemical reactions and the ability of inducing changes in the loop.

(2) Correlation of serological characteristics and the pathological changes.

As is seen in Table 2, serotype which showed the highest rate of the changes was Flexneri 3a followed by 2a, 4a, 1a, 2b and sonnei I. Although Flexneri var. Y showed rather high rate of change, the degree of the change was weak compared to others and I think

the ability of this sero-type to induce changes are the weak one. Sh. flexneri var. X showed no change at all, though the number of the strains tested were small. We isolated the opeque colonies which have weak type-specific antigens and more group antigens from the newly isolated organisms and compared for their ability to induce changes with that of the original strain. The opeque colonies showed a very few positive loops while the latter showed almost 100% positive (Table 3). From this, it seems that the pathogenicity of the organism directly associated with the type-specific antigens, but, as we stated before, the pathogenicity of the organisms also associated with the lac locus, it is conceivable that the locus controlling the pathogenicity is located between the lac locus and the loci controlling the type-specific antigens.

We also sought for the specific antigens which directly associated with the pathogenicity by immunizing rabbits with the pathogenic and non-pathogenic strains of the same sero-type and absorbing the sera with each other to see whether there is any specific antibody. But, so far, we could not find any specific antigen which exist only in the pathogenic strains, by agglutination technique, and we will continue to seek for the specific antigens by the use of available immunochemical methods.

(3) Correlation of the ability to agglutinated by acid and the pathological changes.

As is seen in Table 4, among 45 acid agglutination positive strains 32 strains showed the positive changes in the loop, but also among 45 acid agglutination negative strains 25 showed positive changes. And out of 57 positive loops, 32 were induced by strains agglutinated by acid, and 25 were induced by strains non-agglutinable by acid. Therefore, there is no direct association between acid agglutination and the ability to induce changes in the loop.

As is seen in Table 5, when we isolated the colonies which were agglutinated by acid very weakly and tested for their ability to induce changes in the loop, these strains showed a very weak or no change, therefore, it looks like the ability to agglutinated by acid is associated with the ability to induce changes, but we assume that the difference in ability to induce changes is associated with other changes than the change in the ability to agglutinated by acid.

d. Summary and Discussion.

In the experiments to examine the specific characters of shigella in conjunction with the ability to induce changes in the loop, we could not find any definit evidence but we can conclude from our data that the loci controlling lactose fermentation are closely linked to the loci controlling the virulence of the organisms and the ability to agglutinated by acid is not associated with the ability to induce changes in the loop.

But in the course of our study, we found several interesting facts. The one is that there are organisms in which their biochemical reaction was altered in one particular fermentation reaction and by that they cannot be classified as a shigella, and yet they can induce changes in the loop. This is quite important for a diagnostic point of view. Usually lactose positive organisms are not seek after as a shigella and yet, as in our case, shigella can be found among the lactose positive strains. Therefore, it will be a good excise to pick up lactose positive colonies if patients showed the typical dysentery symptoms and yet no lactose negative organisms could be isolated.

The second is that there is a decrease in the ratio of strains agglutinated by acid among the newly isolated strains. We do not know whether there is any association with the increase in milder dysentery cases or increase in antibiotic resistant strains or not, but, so far as we tested, there is no correlation between the ability of agglutinated by acid and atibiotic resistance.

4. MUTATION OF ORGANISMS IN THE LOOP.

Occasionally the sero-types of the organisms isolated from patients are different at the beginning and at the converescent stages of the disease or are mixed from the biginning. Sometimes it is explained as a mixed infection, and in other cases it is difficult to explain by this mode.

It is well known that shigella is capable of changing its antigens in vitro in the presence of antibody, or by lysogenic conversion. Therefore, it is possible that the similar change can take place in vivo. Also shigella having an altered biochemical reaction can be isolated from patients and we still do not know the cause of the change.

Our purpose is to examine whether a mutation occurs in the loop or not, and if mutants or organisms which have altered biochemical and /or serological characteristics were isolated, to examine the ability of these mutants to induce changes in the loop and to investigate the selection mechanism.

a. Method.

Shigella strains to be tested was inoculated on nutrient agar slant and cultured at 37C for 16 hours. Organisms were harvested in brain heart infusion broth so as to the suspension contains 2-5x10^{8} organisms per milliliter. Half a milliliter (0.5 ml) of the suspension was injected into washed and unwashed loops. After 24 hours of injection, fluid in a positive loop was drawn aseptically-- in case of a negative loop, inside of the loop was washed with sterile saline -- and a serial dilution was made with sterile saline. One tenth (1/10) ml each of the dilution was plated on SS and BTB lactose agar plates and incubated at 37C for 18 hours. All of the lactose negative

colonies appeared on theplates were picked and examined for their biochemical and serological reactions. Ten (10) lactose positive colonies were also examined for their reactions.

b. Result.

The numbers of the organisms recovered from the positive and the negative loops are shown in Table 6.

As is seen in Table 6, the number of the organisms recovered from the positive loops were from 2.4×10^8 to 3.6×10^{10} and that of the negative loops were from 1.0×10^8 to 8.5×10^9 and there was not much difference between the positive and negative loops. But almost all of the organisms isolated from the washed loops were the lactose negative with a few exception, while the organisms isolated from the unwashed loops were the mixture of both the lactose positive and negative organisms in various proportions, regardless of whether the loop showed the pathological changes or not. Therefore, it suggests that whether the pathological change occurs or not is not merely controled by the number of the organisms but other factors, such as character of organisms or resistance of hosts, play an important role.

All of the lactose negative organisms were tested for their biochemical and serological ractions. Unexpectedly about 60-70% of the biochemically shigella organisms did not react with any of the known shigella sera, even after prolonged heating at 121C. Recovery rates of those organisms are shown in Table 7. It is especially interesting that there were two cases that we could not isolated the typical shigella at all.

We, therefore, examined the agglutinin titer of the rabbits and chequed if there was any correlation between the occurence of the non-agglutinable shigella and the presence of antibodies. But so far there seems no relationship at all, that is, rabbits with no serum antibody still excreate the non-agglutinable shigella. Antibodies present in fluid in the loop and their relation to non-agglutinable strains is now under study. We will examine this phenomenon further in the continuing contract period.

Lactose positive organisms which were isolated from unwashed loops were examined for their biochemical as well as serological reactions. There were many lactose positive organisms having a type-specific as well as group antigens of shigella. Some of them differ from shigella only in their lactose fermentation and some of them had only a part of shigella antigens. Most of the organisms having the partial antigens of shigella are biochemically far apart from shigella and belong to E. coli, proteus, klebsiella and other Enterobacteriacea. We tested the ability of those lactose positive organisms to induce changes in the loop. Of 24 strains, which biochemical reaction was changed in one or two fermentation reaction and serologically identical with shigella, 10 showed changes in the loop, while other strains which

biochemical and serological reaction was far apart from shigella showed no change at all.

Therefore, we think that the organisms which have type-specific antigens and differ from shigella in one or two fermentation reaction are derived from mutation of shigella or from the recombination of shigella with other enteric organisms in the loop. But, because we could not isolate the lactose positive shigella from the washed loops we feel that the lactose positive variety of shigella are derived from recombination of shigella with other residential organisms. Therefore, we have concentrated our efforts to the study of interaction of shigella and residential organisms.

5. INTERACTION OF SHIGELLA WITH RESIDENTIAL ORGANISMS.

In the case of dysentery epidemics, there are always people who do not fall ill even when they dischage many shigella organisms in the stool, or are people who do not infected at all. In some cases immunity will play a role, but we cannot explain it only with immunity. It is possible that the residential organisms interact with the invading organisms and inhibit their proliferation, or change their ability to cause disease.

In our early experiments it was shown that the unwashed loop has lower rate of change occuring when compared with the washed loop and, as we shown in the foregoing experiments, from unwashed loops we isolated many lactose positive shigella or other enteric organisms having a common antigen with shigella. These results suggest the possibility of occuring the recombination in the loop.

Our purpose of the experiment is to examine the role of the residential organisms in a loop upon infection as well as a factor which changes the character of invading organisms.

a. Method.

We isolated E. coli from healthy rabbits, from positive and negative loops and tested for their ability to induce changes in the loop. None of them induced change. Therefore, we introduced these strains along with shigella which can induce changes in the loop. The loops were examined after 24 hours whether any change occured or not.

We also used E. coli K-12 Hfr strain to see whether any recombination occurs in the loop or not, because we know this strain can recombine with shigella *in vitro*.

b. Results

Twenty three (23) strains of E. coli were tested and only one of them which was isolated from a positive loop prevented the changes when introduced with Sh. flexneri 3a. In this case 1.4×10^8 of shigella and 3×10^8 of E. coli were introduced into the loop. The number of

the shigella recovered after 24 hours was 8×10^9 and that of the control loop which received shigella alone was 9×10^9 and the control loop showed the positive changes. We repeated the same experiment several times and had the same results. Therefore, this particular strain has some ability to prevent the induction of changes without influencing the proliferation of the organisms. This E. coli is not typable with known sera of E. coli, nor have colicinogenic activity, and there is no particular characteristics which differentiate this strain from other E. coli, therefore, we still do not know why this strain inhibited the induction of the changes.

We have tested the ability of shigella organisms isolated from the same loop to induce changes in the loop. Those organisms did not induce the changes at all, and we thought at first that the organisms have been changed their ability by E. coli, but the organisms isolated from the control loops also lost the ability, therefore, another explanation is neccessary for this phenomenon. We, therefore, isolated the organisms at interval after the inoculation and tested their ability to induce changes, and found that the loss of the ability is due to mutation and selection, i.e., organisms isolated from the loop at the early stage maintain their ability but the organisms isolated from the latter stage lost their ability, even when the organisms were isolated from a positive loop.

In contrast to the above, one of the E. coli which was isolated from a negative loop and itself could not induce any change at all has an ability to enhance the changes when it was introduced with a strain of Sh. flexneri 3a which has a weaker ability to induce changes. This E. coli too has no particular characteristics which can be differentiated from other E. coli. We will investigate this strain in the continueing contract period.

We also introduced E. coli K-12 Hfr strain with shigella and examined whether any recombination takes place in the loop or not. As are seen in the Table 8 , all the loops tested showed a weaker changes compared with the one inoculated with shigella alone.

In case of Sh. flexneri 3a, no lactose negative colony was recovered from the loop, but by the further examination it was found that those lactose positive organisms were not all E. coli, but 70% of them were lactose positive Sh. flexneri 3a. In case of Sh. flexneri 2a, no lactose positive organisms was recovered from two loops out of three loops examined, but those lactose negative organisms were not all shigella but some of them were biochemically shigella without known shigella antigens. From remaining one loop, lactose positive organisms were isolated too, but in this case too, not all lactose positive organisms were E. coli, but some of them were shigella with lactose positive property. In case of Sh. flexneri 2b and Sh. sonnei I, lactose positive organisms were all E. coli, but there were many lactose negative organisms which biochemical reaction was the typical shigella yet did not agglutinated with any of the known shigella antisera.

The mechanism of occurence of a lactose positive shigella and occurence of a lactose negative shigella without shigella antigens can be explained by the genetic recombination, because these phenomena take place in vitro, but the rate of appearing these organisms are the rare event in vitro, therefore, we suspect that some kind of selection mechanism is working in the loop. We will investigate the mechanism in the future.

Shigella strains having the lactose positive property and the shigella without shigella antigens were tested for their ability to induce changes in the loop, but none of them have the ability. Therefore, we still do not know which of the properties -- lactose fermentation and antigens--is associated with the ability to induce changes in the loop. But the probable explanation will be that the loci controlling the lactose fermentation and antigens are closely linked and the locus controlling the ability to induce changes in the loop is located between them.

SUMMARY

1. Shigella having the ability to ferment lactose or sucrose can be isolated from dysentery patients.

2. The lactose positive or sucrose positive shigella are capable of inducing the pathological changes in the loop.

3. The ability of agglutinated by acid in shigella is not associated with the ability of inducing changes in the loop.

4. Organisms having the ability to agglutinated by acid are decreasing in number among the newly isolated shigella.

5. No correlation was found between the decrease in number of organisms having the ability to agglutinated by acid and the increase in antibiotic resistant mutants.

6. The number of organisms recovered from the positive and the negative loops were almost the same.

7. Shigella isolated from early stages of loop and the organisms isolated from later stages of the loop was differ in the ability to induce the pathological changes.

8. Recombination of shigella with residential organisms does occur in the loop, and lactose positive shigella or shigella without shigella antigens can be produced in the loop.

9. There were organisms which inhibited or enhanced the induction of the pathological changes in the loop without acting on the shigella organisms.

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Table 1

The ability of lactose positive shigella to induce changes in the loop.

Strain No.	Days to ferment lactose	Sero-type	Changes of the loop
45	4	Flex. 1b	+++
46	7	"	-
47	6	"	-
48	4	"	-
78	3	Flex. 2a	-
30	3	"	-
82	6	"	±
141	4	Flex. var. Y	-

Table 2

The rate of induction ofpathological changes by shigella sero-type

Sero-type		Changes of the loop +++	++	+	-	Rate of positive loop	%
Flex.	1a	2			1	2/3	66
	1b	1		2	6	3/9	33
	2a	18	18	6	13	42/55	76
	2b	5	1	5	5	11/16	69
	3a	12	7	5	2	24/26	92
	4			1	1	1/2	
var.	X				1	0/1	
var.	Y			3	1	3/4	75
Sonnei	I	5	4	3	3	12/15	80
Total		43	30	25	33	103/138	75

Table 3

Comparison of the rate of induction of pathological changes between opaque and translucent colonies.

Rabbit No.	Translucent colony	Opaque colony
GR 33	+++	+
36	+++	++
37	+++	-
38	+++	-
39	+++	±
60	++	-
Rate of positive	6/6	3/6
% positive	100	50

Table 4

Correlation of the ability to agglutinated by acid and the induction of pathological changes

Sero-type		Positive loop Acid agglutination +	Positive loop Acid agglutination -	Negative loop Acid agglutination +	Negative loop Acid agglutination -
Flex.	1a		2		
	1b		1		4
	2a	14	17	7	7
	2b	3	1		8
	3a	14	3	3	
	4	1		1	
var.	X				1
var.	Y		1	2	
Total		32	25	13	20

Ability to induce changes in the loop:

Strains agglutinated by acid ------ 32/45 = 71%

Strains not agglutinated by acid -- 25/45 = 56%

Table 5

Comparison of the rate of induction of pathological changes between original and acid non-agglutinable mutant

Strain No.	Original strain	Acid non-agglutinable strain
54	++	-
55	++	-
58	+	+
59	+++	-
40	++	-
62	+++	-
63	++	-
64A	+++	-
64C	+++	-
65A	+++	-
65C	++	-
66A	++	-
66C	++	-
70A	++	-
70C	-	-
71A	-	-
71C	++	-
72A	+	-
72C	+	-
73A	+	-
73C	++	++
Rate of positive	19/21	2/21
% positive	90%	9.5%

Sero-type of the strains used are Flex. 3a.

Table 6

The number of total shigella recovered from positive and negative loops

Positive loop				Negative loop			
Sero-type	Rabbit No.	Inoculum size	Number shigella recovered	Sero-type	Rabbit No.	Inoculum size	Number shigella recovered
1a	C27	3.0×10^{8}	6.7×10^{9}	1b	C27	2.0×10^{8}	7.0×10^{9}
"	"	1.4×10^{8}	1.6×10^{9}	"	"	1.2×10^{8}	3.3×10^{9}
1b	C28	2.0×10^{8}	1.5×10^{9}	"	C28	1.6×10^{9}	1.5×10^{9}
2a	C29	1.0×10^{8}	2.3×10^{10}	"	"	2.5×10^{8}	2.9×10^{9}
"	"	3.5×10^{8}	2.4×10^{8}	"	"	2.5×10^{8}	4.5×10^{9}
"	"	3.5×10^{8}	1.1×10^{10}	"	"	7.0×10^{8}	5.5×10^{9}
"	C30	2.0×10^{8}	2.7×10^{9}	"	C29	4.0×10^{8}	6.5×10^{8}
"	"	1.5×10^{8}	10^{10}	"	"	1.5×10^{8}	2.5×10^{9}
"	"	5.0×10^{8}	1.3×10^{10}	2b	C39	4.0×10^{8}	5.0×10^{8}
"	"	1.5×10^{8}	6.3×10^{9}	"	C40	2.5×10^{8}	2.0×10^{9}
"	"	2.5×10^{8}	7.0×10^{9}	"	"	3.0×10^{8}	3.5×10^{9}
2b	C39	2.5×10^{8}	2.0×10^{9}	"	"	3.5×10^{8}	5.0×10^{8}
"	"	5.0×10^{8}	1.3×10^{9}	"	"	2.5×10^{8}	1.0×10^{9}
"	"	2.5×10^{8}	3.6×10^{9}	"	C41	3.0×10^{8}	3.5×10^{9}
"	"	4.5×10^{8}	5.0×10^{9}	3a	"	4.0×10^{8}	8.5×10^{9}
"	"	3.4×10^{8}	4.8×10^{8}	"	"	2.5×10^{8}	5.0×10^{8}
3a	C45	4.0×10^{8}	3.6×10^{10}	4	C47	4.5×10^{8}	1.0×10^{9}
"	C46	3.0×10^{8}	8.5×10^{8}	var.X	"	5.0×10^{8}	1.0×10^{9}
"	"	2.5×10^{8}	4.0×10^{8}	Y	"	1.4×10^{8}	4.0×10^{8}
"	"	3.0×10^{8}	2.4×10^{9}	"	"	1.5×10^{9}	1.0×10^{8}
"	"	3.5×10^{8}	2.0×10^{9}	"	C48	5.0×10^{8}	2.3×10^{9}

Table 7

Rate of appearance of shigella having no known shigella antigens

Rabbit No.	Sero type	Strain No.	Inoculum size	Change of loop	Number lac^- organism	Number lac^+ organism	Rate of shigella without shigella antigens
27a	1a	39	3.0×10^8	+++	6.7×10^9	10^2	1/3
27b	"	40	2.5×10^8	+++	1.6×10^9	50	1/6
29c	2a	51	1.0×10^8	+++	10^6	10^2	1/2
30b	"	55	1.5×10^8	+++	10^{10}	10^3	2/3
30c	"	56	5.5×10^8	+++	1.3×10^{10}	10^2	1/3
39d	2b	102	4.5×10^8	+++	5.0×10^9	10^3	1/3
43c	3a	121	3.5×10^8	++	2.1×10^9	10	2/5
43e	"	123	2.0×10^8	+	5.0×10^8	10^3	1/4
44d	"	127	7.5×10^8	++	1.3×10^9	10^2	3/3
28d	1b	48	7.5×10^8	-	5.5×10^9	10^4	3/3

Rate of appearance of shigella having no known antigens in

a) positive loops 9/24
b) negative loops 1/9

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DOCUMENT CONTROL DATA - R&D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author) Department of Bacteriology, Kitasato Institute Tokyo, Japan	2a. REPORT SECURITY CLASSIFICATION Unclassified 2b. GROUP

3. REPORT TITLE
STUDIES ON EXPERIMENTAL SHIGELLOSIS: SPECIAL REFERENCE TO SPECIAL CHARACTERISTICS OF SHIGELLA SPECIES AND THEIR ACTION UPON LIGATED LOOP (U)

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
Final Report, 15 April 1964 - 14 April 1965

5. AUTHOR(S) (Last name, first name, initial)
Ghoda, Akira

6. REPORT DATE 2 October 1965	7a. TOTAL NO. OF PAGES 22	7b. NO. OF REFS 11
8a. CONTRACT OR GRANT NO. DA-92-557-FEC-37307 b. PROJECT NO. 2N01,501B71D c. Task 00 007FE d.	9a. ORIGINATOR'S REPORT NUMBER(S) J-187 9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	

10. AVAILABILITY/LIMITATION NOTICES
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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY U.S. Army R&D Group (Far East) APO San Francisco 96343

13. ABSTRACT

The serological, biochemical and enzymic specificities of shigella associated with virulence were sought after in conjunction with the ability to induce changes in the ligated intestinal loop of rabbits. The lactose or sucrose positive shigella were isolated from patients and the correlation of the ability to ferment lactose and sucrose and the ability to induce changes in the loop were examined. The ability to ferment lactose seems to associated with virulence but antigens seems more closely associated with virulence. The ability to be agglutinated by acid which was said to be associated with virulence was not associated with the ability to induce changes. The pathological changes were not associated with the number of shigella recovered after 24 hours of inoculation, if the number exceeded 10⁸. Shigella was mutated to non-virulent mutants several hours after inoculation and some shigella recombined with residential organisms and became lactose positive or shigella without shigella antigens. These organisms had weaker ability to induce changes. But on the other hand, there were residential organisms which enhanced the pathological changes in the loop. (Author)

DD FORM 1 JAN 64 1473

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14. KEY WORDS	LINK A		LINK B		LINK C	
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Histology						
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